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Poster Presentation

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Comparative analysis of *E. coli* inclusion bodies and thermal protein aggregates

Núria González-Montalban^{*1,2}, Elena García-Fruitós^{1,2}, Salvador Ventura^{1,3}, Anna Arís^{1,2} and Antonio Villaverde^{1,2}

Address: ¹Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain, ²Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain and ³Departament de Bioquímica i de Biologia Molecular, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain

* Corresponding author

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Background

In bacteria, inclusion bodies are commonly observed during overexpression of plasmid-encoded recombinant genes, and represent a great matter of concern in biotechnology [1]. Bacterial inclusion bodies are also connected to the protein quality control [2] and to the prevention of cytotoxicity associated to aberrantly folded proteins [3,4]. On the other hand, these protein aggregates are dynamic structures, since they grow as the result of an unbalanced equilibrium between protein deposition and removal [5,2]. Therefore, there is not any physiological evidence of bacterial inclusion bodies being structures well organized to facilitate embedded protein removal by chaperones or proteases. We have comparatively analyzed the molecular organization and dynamism of a recombinant *E. coli* β -galactosidase and its derivative VP1LAC [6] when either deposited as inclusion bodies or as aggregates resulting from *in vivo* thermal denaturation in a laboratory wild type strain *E. coli* MC4100 and its derivatives DnaK and GroEL44 (namely JGT20 and BB4565, respectively). The expression of both *lacZ* and *VP1LAC* genes is triggered by temperature up shift from 28°C to 42°C.

Results

A small part of the recombinant β -galactosidase present in the cell (~5%) was found in the insoluble cell fraction as a result of a heat shock at 42°C and remained nearly constant during the 3-hours heat shock. However, a progressively higher fraction of VP1LAC (up to 45% at 3 h)

occurred as inclusion bodies (data not shown). Nevertheless, this compositional evolution was parallel to a structural evolution (see Figure 1) since polypeptides embedded in inclusion bodies undergo a continuous formation of extended, intermolecular β -sheet structure. This was deduced from the evolution of the bands approximately 1627 cm⁻¹ and 1692 cm⁻¹ relative to that at 1652 cm⁻¹. On the other hand, recombinant β -galactosidase only represents around 3% of the protein species found in insoluble fraction, while VP1LAC accounted for 90% of the inclusion body material. In fact, inclusion bodies were enriched with VP1LAC species, especially in those native-like forms (see Figure 1) peaking approximately at 1638–1640 cm⁻¹.

The formation of β -galactosidase thermal aggregates and VP1LAC inclusion bodies was explored in absence of either the main cytoplasmatic chaperones DnaK (JGT20) and GroEL (BB4565). As expected (see Table 1), the soluble β -galactosidase was more active than the soluble engineered version VP1LAC. Despite this fact, protein aggregated as inclusion bodies was much more active (from 2 to 8 fold) than that occurring in thermal aggregates (up to 10 fold in wild type cells), indicating a higher occurrence of properly folded protein. While, GroEL seems to be fairly relevant, this event it is clearly depending on DnaK, as in JGT20, insoluble VP1LAC is less active than insoluble β -galactosidase.

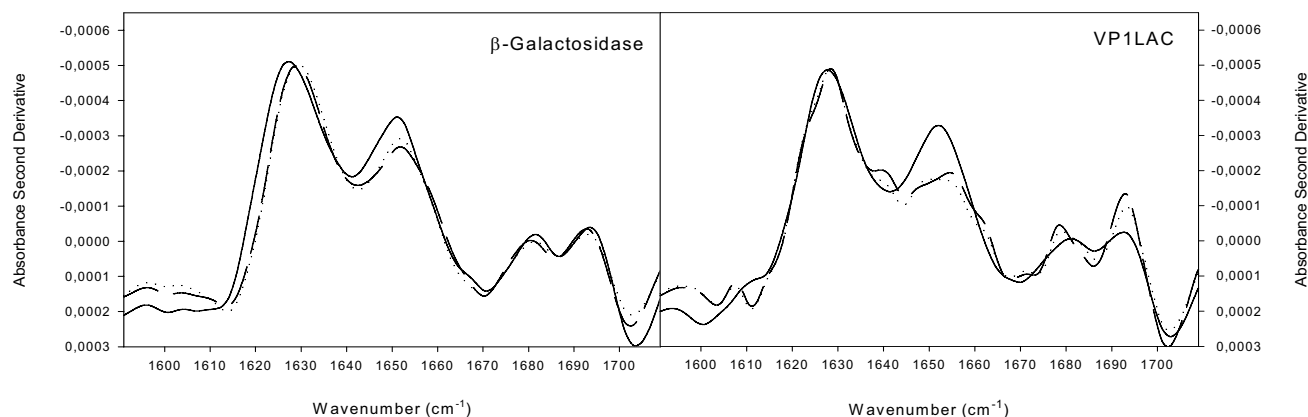


Figure 1
FTIR of β -galactosidase aggregates (left) and VP1LAC inclusion bodies (right) formed during 1 hour (continuous), 3 hours (dotted) or 5 hours (dashed).

Table 1: Specific activity (in U/ng) of β -galactosidase and its derivative VP1LAC produced in different strains, in the soluble and insoluble fractions.

Strain	<u>Solublefraction</u>	<u>Insolublefraction</u>
MC4100/pJCO46	628.2 \pm 40.5	6.3 \pm 0.3
MC4100/pJVP1LAC	234.1 \pm 52.9	65.2 \pm 19.4
BB4565/pJCO46	689.7 \pm 164.9	63.6 \pm 2.2
BB4565/pJVP1LAC	230.2 \pm 25.7	129.6 \pm 45.9
JGT20/pJCO46	888.9 \pm 179.3	175.2 \pm 34.9
JGT20/pJVP1LAC	12.5 \pm 3.8	10.3 \pm 6.3

Conclusion

Thermal denaturation of β -galactosidase results in the formation of heterogeneous aggregates that are rather stable in composition during the heat shock stress. On the contrary, protein deposition as inclusion bodies renders homogeneous but strongly evolving structures. In this context, the specific activity of enzyme-based inclusion bodies is much higher than in the equivalent thermal aggregates, by a mechanism that might be controlled by the chaperone DnaK. Protein deposition as inclusion bodies is then a cell driven complex process through which misfolded protein forms but also functionally competent polypeptides are efficiently packaged.

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